National Organic Standards Board
Materials Subcommittee
Excluded Methods Discussion Document
Plant Breeding Techniques "TBD List"
February 14, 2023

Summary of Request:

The Materials Subcommittee is seeking feedback on the remaining To Be Determined (TBD) List in the Excluded Methods Plant Breeding Techniques discussion document. The goal is to determine whether the techniques are excluded methods under the legal definition at 205.2, OFPA criteria, as well as previous NOSB recommendations. . The Materials Subcommittee has requested a limited scope TR for induced mutagenesis in order to facilitate the work and has included questions for stakeholder feedback in this discussion document.

Summary of Review:

Draft Definitions

TILLING (Targeted Induced Local Lesions in Genomes):

This technique has two parts:

First, mutations are induced in seeds by treating them with mutagenic chemicals, or, less commonly, using environmental stresses or radiation. These provide an increased frequency of point mutations distributed randomly in the genome. In Eco-TILLING, mutagens are not used. Genetic diversity is obtained by using diverse seed sources.

Second, samples are taken from seedlings grown out from step one, and tested to find those with mutations in a targeted location within the plant genome. Desired seedlings can then be used for breeding.

Double Haploid (DH):

Double haploid is a genotype of two identical chromosomes from spontaneous or artificial doubling, which produces pure homozygous or inbred lines. This greatly expedites breeding procedures, slimming a 5-or-6-year task to only one generation. To date, maize is the predominant crop bred using this technique.

There are two methods to produce DH plants. In-vivo and in-vitro with the use of synthetic chemicals to double chromosomes.

Firstly, in-vivo methods accomplish double haploidy with cross pollination techniques using irradiated pollen or haploid inductor or inducer lines. Megaspores/ovule cells can be induced with pollen of the inductor lines to develop haploid embryos without recombination of genes. The haploid embryo can spontaneously double its chromosomes to become homozygous double haploid plants. Double haploidy can be obtained all in-vivo.

Secondly, in-vitro methods are plant tissue cultures (often times treated with phytohormones to stimulate growth) of haploid cells from either microspores/pollen in androgenesis or megaspores/ovules in gynogenesis which differentiate into haploid embryos then haploid plants. These haploid plants will duplicate their chromosomes spontaneously or with the application of colchicine, a synthetic chemical resulting in a homozygous double haploid plant.

Induced Mutagenesis:

Mutagenesis is a process by which the genetic information of an organism is changed by the production of a mutation(a). The agents that cause mutation are called mutagens(b). Mutation induced by mutagens is called induced mutation.

> [a]*In biology, a mutation is an alteration in the nucleic acid sequence of the genome of an organism, virus, or extrachromosomal DNA)*
> [b]*In genetics, a **mutagen** is a physical or chemical agent that permanently changes genetic material, usually DNA, in an organism and thus increases the frequency of mutations above the natural background level.*

Mutagenesis may occur spontaneously in nature, or as a result of exposure to mutagens. It can also be achieved experimentally using laboratory procedures. A mutagen is a mutation-causing agent, be it chemical or physical, which results in an increased rate of mutations in an organism's genetic code. In nature mutagenesis can lead to cancer and various heritable diseases, and it is also a driving force of evolution.

Transposons:

A chromosomal segment that can undergo transposition, especially a segment of bacterial DNA that can be translocated as a whole between chromosomal, phage, and plasmid DNA in the absence of a complementary sequence in the host DNA. Also called jumping gene.

A transposable element (TE, transposon, or jumping gene) is a nucleic acid sequence in DNA that can change its position within a genome, sometimes creating or reversing mutations and altering the cell's genetic identity and genome-size.

Questions:

1. Please provide feedback on the draft definitions along with relevant peer reviewed scientific sources.
2. In the use of each technique, please describe the individual steps and the application of prohibited substances or excluded methods that may be employed in that step towards creating new varieties of plants or animals, if applicable.
3. For each technique, describe/list plant varieties, clones, etc. which may have been developed and are currently being used in organic systems.

To Be Determined "TBD list":

Terminology				
Method and synonyms	**Types**	**Excluded Methods**	**Criteria Used**	**Notes**
TILLING	Eco-TILLING	*TBD*		Stands for "Targeted Induced Local Lesions in Genomes." Standard TILLING includes induced mutagenesis. Eco-TILLING does not.
Doubled Haploid Technology (DHT)		*TBD*		There are several ways to make double haploids, and some do not involve genetic engineering while some do. It is difficult or impossible to detect DHT with tests.
Induced Mutagenesis		*TBD*		Induced mutagenesis developed through exposure to UV light, chemicals, irradiation or other stress.
Transposons		*TBD*		Produced from chemicals, ultraviolet radiation, or other synthetic activities.

Excluded Methods:

Method and synonyms	Types	Excluded Methods	Criteria Applied	Notes
Targeted genetic modification (TagMo) syn. Synthetic gene technologies syn. Genome engineering syn. Gene editing syn. Gene targeting	Sequence-specific nucleases (SSNs) Meganucleases Zinc finger nuclease (ZFN) Mutagenesis via Oligonucleotides CRISPR-Cas system (Clustered regularly interspaced short palindromic repeats) and associated protein genes TALENs (Transcription activator-like effector nucleases) Oligonucleotide directed mutagenesis (ODM) Rapid Trait Development System	YES	1, 3, 4	Most of these new techniques are not regulated by USDA and are currently difficult to determine through testing.
Gene Silencing	RNA-dependent DNA methylation (RdDM) Silencing via RNAi pathway RNAi pesticides	YES	1, 2, 4	
Accelerated plant breeding techniques	Reverse Breeding Genome Elimination FasTrack Fast flowering	YES	1, 2, 4	These may pose an enforcement problem for organics because they are not detectable in tests.
Synthetic Biology	Creating new DNA sequences Synthetic chromosomes Engineered biological functions and systems	YES	1, 3, 4	
Cloned animals and offspring	Somatic nuclear transfer	YES	1, 3	
Plastid transformation		YES	1, 3, 4	
Cisgenesis	The gene modification of a recipient plant with a natural gene from a crossable-sexually compatible-plant. The introduced gene includes its introns and is flanked by its native promoter and terminator in the normal-sense orientation.	YES	1, 3, 4	Even though the genetic manipulation may be within the same species, this method of gene insertion can create characteristics that are not possible within that individual with natural processes; it can have unintended consequences.

Method and synonyms	Types	Excluded Methods	Criteria Applied	Notes
Intragenesis	The full or partial coding of DNA sequences of genes originating from the sexually compatible gene pool of the recipient plant and arranged in sense or antisense orientation. In addition, the promoter, spacer, and terminator may originate from a sexually compatible gene pool of the recipient plant.	YES	1, 3, 4	Even though the genetic manipulation may be within the same species, this method of gene rearrangement can create characteristics that are not possible within that individual with natural processes; it can have unintended consequences.
Agro-infiltration		YES	1, 3, 4	*In vitro* nucleic acids are introduced to plant leaves to be infiltrated into them. The resulting plants could not have been achieved through natural processes and are a manipulation of the genetic code within the nucleus of the organism.
Transposons-Developed via use of in vitro nucleic acid techniques		YES	1,3,4	Does not include transposons developed through environmental stress such as heat, drought or cold.
Induced Mutagenesis		YES	1	Developed through in vitro nucleic acid techniques does not include mutagenesis developed through exposure to UV light, chemicals, irradiation, or other stress-causing activities.
Cell and Protoplast Fusion	Donor and/or recipient cells are outside taxonomic plant family; and/or recombinant DNA technology is employed	YES	Terms Defined 205.2	See NOP Policy Memo 13-1.

Methods Allowed:

Method and synonyms	Types	Excluded Methods	Criteria Applied	Notes
Marker Assisted Selection		NO		
Transduction		NO		
Embryo rescue in plants		NO		IFOAM's 2018 position paper on Techniques in Organic Systems considers this technique compatible with organic systems.
Embryo transfer, or embryo rescue, in animals		NO		*use of hormones not allowed in recipient animals.
Transposons		NO		Developed through environmental stress, such as heat, drought, or cold.
Cell and Protoplast Fusion	Recipient and/or donor cells are within the same taxonomic plant family; must be achieved without recombinant DNA technology	NO		NOP Policy Memo 13-1; Definition of Modern Biotechnology

Subcommittee Vote:

Motion to accept the discussion document on excluded methods TBD List
Motion by: Mindee Jeffery
Seconded by: Brian Caldwell
Yes: 5 No: 0 Abstain: 0 Recuse: 0 Absent: 1